Synopsis of the thesis entitled

PHARMACOLOGICAL INVESTIGATIONS ON ANTI-INFLAMMATORY, HEPATOPROTECTIVE AND ANTIMICROBIAL ACTIVITIES OF SOME SELECTED INDIAN MEDICINAL PLANTS

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By

SAMBASIVA RAO ETHADI

M. Pharm.,PGDMB.

Under the Supervision of

Guide

Prof. P.RAJESWARA RAO

M.Pharm., Ph.D.

Co-Guide

Prof. B.GANGA RAO

M.Pharm., Ph.D. (India), Ph.D.(U.K.), P.D.F.(Australia)

A.U College of Pharmaceutical Sciences

Andhra University

Visakhapatnam – 530 003

Andhra Pradesh, INDIA

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Chapter I

Introduction:

An Introduction to health and disease, and evaluation of various remedial measures since time immemorial, recognition of plants as a source of drugs, the aims and objectives of the present study are dealt in the Chapter I.

Chapter II

Review of Literature

This chapter deals with the morphological characters of the selected plants (*Cleome chelidonii, Gynandropsis gynandra* and *Heliotropium indicum*) with an updated review of phytoconstituents and pharmacological activities that have been reported by various researchers , Scope and objective of the present investigation are described in Chapter II.

Chapter III

Phytochemical Screening:

This chapter describe the preliminary phytochemical screening for the presence various phytoconstituents in three selected plant extracts and quantification of total phenolic and alkaloidal content.

Cleome chelidonii root

Qualitative phytochemical screening of different extracts of *Cleome chelidonii* revealed the presence of steroids, terpenoids, glycosides, tannins,

alkaloids, flavonoids, phenols, oils and carbohydrates and showed negative to quinines and amino acids. Methanolic extract showed positive to oils and saponins and the remaining extracts showed negative to oils and saponins.

The phenolic content of various extracts of *Cleome chelidonii* root was ranging from 14.56±0.86 to 38.95±0.39 (mg per gm). The methanolic extract possesses more phenolic content (38.95±0.39 mg per gm) than other extracts. Alkaloid content in extracts was ranging from 16.55±0.23 to 36.86±0.52 (mg per gm). The methanolic extract contains more alkaloid content (36.86±0.52 mg per gm) than other extracts.

Gynandropsis gynandra

Qualitative Phytochemical screening for different extracts of *Gynandropsis gynandra* whole plant revealed the presence of steroids, terpenoids, glycosides, tannins, alkaloids, flavonoids, phenols and carbohydrates. The methanolic, hydro-alcoholic extracts contain saponins and the hexane, ethyl acetate extracts showed negative to saponins, oils and the all extracts are showed negative to quinines, amino acids.

The phenolic content of various extracts of *Gynandropsis gynandra* extracts were ranging from 13.21±0.66 to 72.80±0.22 (mg per gm). The Hydro-alcoholic extract contains more phenolic content (72.80±0.22 mg per gm) than other extracts. The alkaloidal content of extracts were ranging from 8.91±0.10 to 16.68±0.21 (mg per gm) and the methanolic extract contains

more alkaloidal content (16.68±0.21 mg per gm) than other extracts.

Heliotropium indicum

Qualitative chemical tests indicated that the hydro-alcoholic extract *H. indicum* whole plant showed positive test for Steroids, Triterpenoids, Saponins, Flavonoids, Carbohydrates, Glycosides, Amino acids and oils. The methanolic extract of *H. indicum* showed positive test Triterpenoids, Saponins, Carbohydrates, Glycosides, phenols, flavanoids, Amino acids and oils. The ethyl acetate extract of *H.indicum* showed positive for Steroids, Saponins, Flavonoids, Carbohydrates, Glycosides, Amino acids and oils and the hexane extract of *H. indicum* showed positive to Saponins, Carbohydrates and Amino acids.

The phenolic content in hydro-alcoholic crude extract, methanolic, ethyl acetate and hexane extracts of *H. indicum* whole plant was found to be 2.32±0.25, 9.76±0.11, 2.96±0.55 and 5.49±0.17 mg per gm respectively. Among the selected plant extracts ethyl acetate extract of *H. indicum* showed high phenolic content. The alkaloid content in hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *H. indicum* was found to be 10.56±0.55, 12.61±0.22, 7.32±0.35 and 8.96±0.16 mg per gm respectively. Among the selected extracts, ethyl acetate extract of *H. indicum* contains more phenolic content.

Chapter IV

Acute toxicity study

The acute toxicity study was conducted for Hydro-alc, methanolic, ethyl acetate and hexane extracts of *Cleome chelidonii* roots, whole plant of *Gynandropsis gynandra* and *Helitropium indicum* as per OECD guidelines 420 (OECD.2001).

Acute toxicity studies in mice revealed that the extracts up to 2000 mg/ kg produced no sign of toxicity or mortality.

Chapter V

In-vitro Antioxidant activity:

This chapter described introduction to free radicals, antioxidants and list of plants with antioxidant activity. The experimental procedures for *in vitro* antioxidant activity of the extracts of the selected plants i.e *C.chelidonii* root, whole plant of *G.gynandra, H.indicum* and known antioxidant Ascorbic acid (standard) against Superoxide, Hydroxyl, DPPH radicals and the results and discussion.

Superoxide radical scavenging activity:

Cleome chelidonii

In the present study, the hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *C.chelidonii* root were found to possess concentration dependent scavenging activity on superoxide radical scavenging activity. The mean IC_{50} values for superoxide radical scavenging activity of hydro- alcoholic

extract, methanolic, ethyl acetate and hexane extracts of *C.chelidonii* root were found to be 130.00±1.4, 101.00±1.2, 177.00±2.2 and 552.5±3.4 µg respectively. The mean IC_{50} value of ascorbic acid was found to be 53.5±1.2 µg.

Gynandropsis gynandra

The hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *G.gynadra* whole palnt were found to possess dose dependent superoxide radical scavenging activity. The mean IC_{50} values for superoxide radical scavenging activity of hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *G.gynandra* were found to be 150.5±1.5, 126.5±1.3, 259.2±2.1 and 575.0±2.3 µg respectively.

Heliotropium indicum

The hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *H.indicum* whole plant were found to possess concentration dependent superoxide radical scavenging activity. The mean IC_{50} values for superoxide radical scavenging activity of hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *H.indicum* whole plant were found to be 139.5±2.1, 110.2±1.5, 195.0±1.5 and 294.5±2.2 µg respectively.

Hydroxyl radical scavenging activity:

Cleome chelidonii

The hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *C.chelidonii* roots were found to possess concentration dependent scavenging activity on hydroxyl radicals. The mean IC_{50} values for hydroxyl radical scavenging activity of hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *C.chelidonii* roots were found to be 193.00±2.2, 136.5±1.2, 353.00±3.1 and 544.00±2.5 µg respectively. The mean IC_{50} value of ascorbic acid was found to be 67.8±2.3 µg.

Gynandropsis gynandra

The hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *G.gynandra* whole plant were found to possess concentration dependent scavenging activity on hydroxyl radicals.The mean IC_{50} values for hydroxyl radical scavenging activity of hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *G.gynandra* whole plant were found to be 226.5±2.1, 164.3±1.8, 452.0±2.5 and 709.5±3.2 µg respectively.

Heliotropium indicum

The hydro alcoholic extract, methanolic, ethyl acetate and hexane extracts of *H.indicum* whole plant were found to possess concentration dependent scavenging activity on hydroxyl radicals.The mean IC_{50} values for hydroxyl radical of hydro-alcoholic extract , methanolic, ethyl acetate and hexane extracts of *H.indicum* were found to be 195.2±1.6, 96.1±1.5, 249.5±2.3 and 387.5±2.5 µg respectively. The mean IC_{50} value of ascorbic

acid was found to be 67.8±2.3 µg.

DPPH radical scavenging activity

Cleome chelidonii

The hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *C.chelidonii* root were found to possess concentration dependent scavenging activity on DPPH radicals. The mean IC_{50} values for DPPH radical scavenging activity of hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *C.chelidonii* were found to be 99.2±1.4, 74.8±1.4, 181.00±1.2 and 307.00±2.4 µg respectively. The mean IC_{50} value of ascorbic acid was found to be 18.5±1.5 µg.

Gynandropsis gynandra

The hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *G.gynandra* whole plant were found to possess concentration dependent scavenging activity on DPPH radicals. The mean IC_{50} values for DPPH radical scavenging activity of hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *G.gynandra* were found to be 108.25±2.3, 87.9±1.1, 239.4±2.3 and 340.0±2.2 µg respectively.

Heliotropium indicum

The hydro-alcoholic (Ethanol 70% v/v) extract, methanolic, ethyl acetate and hexane extracts of *H.indicum* were found to possess dose dependent scavenging activity on DPPH radicals. The mean IC_{50} values

for DPPH radical scavenging activity of hydro-alcoholic extract, methanolic, ethyl acetate and hexane extracts of *H.indicum* were found to be 87.9±1.2, 69.7±1.5, 119.2±1.2 and 307.0±2.3 µg respectively.

Among the extracts, the methanolic extract of *C.chelidonii root* showed better free radical scavenging activity against Superoxide, Hydroxyl and DPPH free radicals. The order of activity as follows: Ascorbic acid >Methanolic extract >Hydro-alcoholic extract > Ethyl acetate extract> Hexane extract.

Among the extracts, the methanolic extract of *G.gynandra* showed better free radical scavenging activity against Superoxide, Hydroxyl and DPPH free radicals. The order of activity as follows: Ascorbic acid>Methanolic extract >Hydro-alcoholic extract > Ethyl acetate extract> Hexane extract.

Among the extracts, the methanolic extract of *H.indicum* showed better free radical scavenging activity against Superoxide, Hydroxyl and DPPH free radicals. The order of activity as follows: Ascorbic acid>Methanolic extract >Hydro-alcoholic extract > Ethyl acetate extract> Hexane extract.

Chapter VI

Anti-inflammatory Activity:

This chapter describes the anti-inflammatory activity of *C.chelidonii* root*,* *G.gynandra* and *H.indicum* whole plant extracts in carrageenan induced paw oedema in rats.

Since methanolic extracts have produced maximum activity than hydro-alcoholic, ethyl acetate and hexane extracts. The results were expressed as maximal paw oedema (maximal peak during the 6 h) and as total paw oedema (area under the time-course curve) and presented as Mean ± S.E.M., n=6.

C.chelidonii root

The Indomethacin at a dose of 5 mg/kg and hydro-alcoholic crude extract of *C.chelidonii* root at doses 100, 200 & 400 mg/kg significantly inhibited the maximal oedema response and the percentage inhibition was found to be 62.92±1.5, 37.41±0.8, 44.65±1.2 and 50.28±1.2% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 68.4±1.2, 45.71±0.8, 47.81±1.3 and 51.25±1.1% respectively over 6 h when compared to the control group treated with drug vehicle.

Methanolic extract of *C.chelidonii* significantly inhibited the maximal oedema response and perecentage inhibition was found to be 38.91±0.5, 47.76±1.1 and 53.39±1.2% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 47.15±1.2, 49.68±0.5 and 53.32±0.6% respectively over 6 h when compared to the control group treated with drug vehicle.

Ethyl acetate extract of *C.chelidonii* root significantly inhibited the maximal oedema response and percentage inhibition was found to be 34.92±1.1, 43.08±0.6 and 48.65±1.3% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 44.1±1.2, 46.04±0.5 and 49.45±1.2% respectively over 6 h when compared to the control group treated with drug vehicle.

Hexane extract of *C.chelidonii* root significantly inhibited the maximal oedema response and percentage inhibition was found to be 32.58±1.2, 36.92±1.1 and 44.17±1.2% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 41.55±1.2, 42.66±1.4 and 46.23±1.5% respectively over 6 h when compared to the control group treated with drug vehicle.

G.gynandra

The Indomethacin at a dose of 5 mg/kgand hydro-alcoholic extract of *Gynandropsis gynandra* whole plant significantly inhibited the maximal oedema response and the percentage inhibition was found to be 62.92±1.5, 23.68±1.4, 31.09 ±2.1 and 34.63±2.4% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 68.4±1.2, 34.5±1.1, 39.94±1.5 and 44.82±2.1% respectively over 6 h when compared to the control group treated with drug vehicle.

Methanolic extract of *Gynandropsis gynandra* whole plant significantly inhibited the maximal oedema response and the percentage inhibition was found to be 29.52±1.1, 34.62±1.2 and 38.66±1.5% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 39.15±1.5, 43.02±2.1 and 46.86±2.1% respectively over 6 h when compared to the control group treated with drug vehicle.

Ethyl acetate extract of *Gynandropsis gynandra* whole plant significantly inhibited the maximal oedema response and the percentage inhibition was found to be 17.81±0.5, 22.95±1.2 and 28.9±1.4% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 30.5±1.2, 35.22±1.1 and 39.84±1.5% respectively over 6 h when compared to the control group treated with drug vehicle.

Hexane extract of *Gynandropsis gynandra* whole plant significantly inhibited the maximal oedema response and the percentage inhibition was found to be 16.2±0.5, 21.11±1.2 and 26.41±1.5% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 28.23±1.2, 32.86±1.4 and 37.09±2.1% respectively over 6 h when compared to the control group treated with drug vehicle.

H.indicum

The Indomethacin and hydro-alcoholic extract of *Heliotropium indicum* significantly inhibited the maximal oedema response and the percentage inhibition was found to be 62.92±1.5, 30.84±0.8, 34.22±1.2 and 36.24±1.4% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 68.4±1.2, 40.53±1.5, 44.27±1.4 and 47.81±1.5% respectively over 6 h when compared to the control group treated with drug vehicle.

Methanolic extract of *Heliotropium indicum* significantly inhibited the maximal oedema response and the percentage inhibition was found to be 33.73±0.8,37.45±1.2 and 41.45±1.5% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 40.92±1.6, 45.35±1.5 and 49.51±1.5% respectively over 6 h when compared to the control group treated with drug vehicle.

Ethyl acetate extract of *Heliotropium indicum* significantly inhibited the maximal oedema response and the percentage inhibition was found to be 23.6±0.5, 28.36±1.2 and 32.26±1.5% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 35.45±1.5, 39.91±1.2 and 43.51±1.5% respectively over 6 h when compared to the control group treated with drug vehicle.

Hexane extract of *Heliotropium indicum* significantly inhibited the maximal oedema response and the perecentage inhibition was found to be 19.43±0.5, 24.64±1.2 and 29.58±1.5% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 34.43±1.8, 36.86±1.5 and 40.11±1.5% respectively over 6 h when compared to the control group treated with drug vehicle.

The percentage inhibition of the maximal paw oedema during 6 h for the hydro-alcoholic (Ethanol 70% v/v) extracts of *C.chelidonii, G.gynandropsis* and *H.indicum* at 400 mg/kg were in the following order *C.chelidonii* > *H.indicum* > *G.gynandropsis*.

The percentage inhibition of the maximal paw oedema during 6 h for the methanolic extracts of *C.chelidonii, G.gynandropsis* and *H.indicum* at 400 mg/kg were in the following order *C.chelidonii* > *H.indicum* > *G.gynandropsis*.

The percentage inhibition of the maximal paw oedema during 6 h for the ethyl acetate extracts of *C.chelidonii, G.gynandropsis* and *H.indicum* at 400 mg/kg were in the following order *C.chelidonii* > *H.indicum* > *G.gynandropsis*.

The percentage inhibition of the maximal paw oedema during 6 h for the hexane extracts of *C.chelidonii, G.gynandropsis* and *H.indicum* at 400 mg/kg were in the following order *C.chelidonii* > *H.indicum* > *G.gynandropsis*.

Among the selected three plant extracts, methanolic extracts of *C.chelidonii* root, whole plant of *G.gynadropis* and *H.indicum* produced highly significant reduction of paw oedema than Hydro-alcoholic, ethyl acetate and hexane extracts.

Chapter VII

Hepatoprotective activity:

This chapter decribes the hepatoprotective activity of the selected herbal drugs tested in rats against CCl_4 induced hepatotoxicity at three different doses (100 mg, 200 mg, 400 mg/kg) prophylactically by measuring serum SGOT, SGPT, ALP & T.BIL levels. An increase in the levels of biochemical parameters is a sensitive index for hepatic damage.

The standard and test group animals were treated with 50 mg/kg dose of Silymarin and 100, 200, 400 mg/kg doses of hydro-alcoholic, methanolic, ethylacetate and hexane extracts of *C.chelidonii*, *G.gynandropsis*, *H.indicum* for 6 days. On 6^{th} day, 1hr after treatment with standard drug and selected plant extracts, the animals were intoxicated with CCl_4 in liquid paraffin (1:1 v/v,0.75 ml of CCl_4/kg, i.p.). Serum was separated by centrifugation at 37 °C and used for estimation of various biochemical parameters. Biochemical parameters like Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) , Serum alkaline phosphatase (ALP) ,Serum Total bilirubin (T.Bil) were estimated by using commercial reagent kits in Autoanalyzer (RM4000, Biochemical systems International, Italy).

All the selected plant extracts showed a dose dependent hepatoprotection against CCl_4 induced intoxication. Among the

extracts methanolic extracts of the selected plants produced maximum hepatoprotection at a dose of 400 mg/kg and based on SGPT levels the range of percentage protection offered by the methanolic extracts found to be lying in the range of 71.45% to 81.02%. The order of percentage protection as follows *Cleome chelidonii* root> *Heliotropium indicum*> *Gynandropsis gynandra.* Thus the study clearly indicates the selected three plants possess hepatoprotective activity and the protection produced by the extracts may be due to their free radical scavenging activities.

The percentage protection to the liver damage produced by the test preparation was calculated as per the following equation.

% Protection = 100 x *(SGOT/SGPT/ALP/T.BIL* values of CCl_4 control − *SGOT/SGPT/ALP/T.BIL* values of treatment) /(*SGOT/SGPT/ALP/T.BIL* values of CCl_4 control - *SGOT/ SGPT/ALP/T.BIL* values of before treatment on 6th day).

The percentage protection offered by silymarin and methanolic extracts of selected plants based on SGPT levels on 7th day after treatment was as follows:

Herbal extract	**Dose (% protection)**
Silymarin	50 mg/kg (90.40%)

Methanolic. Ext. of *C.chelidonii* root

100 mg/kg (69.27%), 200 mg/kg (74.05%) and 400 mg/kg (81.02%)

Methanolic. Ext.of *G.gynandra*

100 mg/kg (60.77%), 200 mg/kg (66.25%) and 400 mg/kg (71.45%)

Methanolic. Ext. of *H.indicum*

100 mg/kg (63.75%), 200 mg/kg (69.76%) and 400 mg

Chapter VIII

Anti - microbial Activity:

This chapter includes plant extracts that were screened for antimicrobial activity against various Gram +ve bacteria and Gram -ve bacteria. Anti microbial screening of the plant extracts was carried out by the cup plate method.

All the selected three plants (including different extracts) that were screened for antimicrobial activity against various Gram +ve bacteria and Gram -ve bacteria. Anti microbial screening of the plant extracts was carried out by the cup plate method.

All the extracts at a concentration of 150 µg, 300 µg, 600 µg and 1200 µg per each cup exhibited antibacterial activity against one or other organisms in dose dependent manner.

Cleome chelidonii:

Among all the tested extracts, methanolic and Hydro-alcoholic (Ethanol 70%v/v) extracts of *Cleome chelidonii* root have shown significant anti bacterial activity as compared to that of hexane, ethyl acetate extracts. The extracts showed good zone of inhibition against Gram negative bacteria than Gram positive bacteria.

Hexane extract produced mild zones of inhibition against bacterial strains compared with other extracts. It showed zone of inhibition against one Gram +ve (*Bacillus megaterium*) and two gram –ve (*Pseudomonas aeruginosa* and *Klebsiella pneumonia*) bacterial strains at a concentration of 150µg/cup and maximum zone of inhibition against *Klebsiella pneumonia*, *Streptococcus pneumonia* was found to be 9mm at a concentration of 1200 µg/cup.

Hydro-alcoholic and Ethyl acetate extracts showed moderate zones of inhibition on tested bacterial strains. Hydro-alcoholic extract had showed zone of inhibition showed maximum zone of inhibition (13mm) on *Pseudomonas aeruginosa* at a concentration of 1200 µg/cup and the Ethyl acetate extract showed maximum zone of inhibition (12mm) on *Pseudomonas aeruginosa*

and *Klebsiella pneumoniae* at a concentration of 1200µg/cup.

The methanol extract showed better activity against tested bacterial strains compared to other extracts and showed maximum zones of inhibition (17mm) on *Klebsiella pneumoniae* at a concentration of 1200 µg/cup.

Gynandropsis gynandra

All the tested extracts at different concentrations have shown significant antibacterial activity against gram –ve organisms than gram +ve organisms along with standard drug.

Hexane showed highest zone of inhibition (16mm) against *Pseudomonas aeruginosa* at a concentration of 1200µg/cup.Ethyl Acetate and Hydro-alcoholic (Ethanol 70% v/v) extracts showed moderate zones of inhibition on tested bacterial strains. Ethyl Acetate extract showed maximum zone of inhibition (15mm) on *Escherichia coli* at a concentration of 1200µg/ cup. Hydro-alcoholic extract showed highest zone of inhibition (13mm) *Pseudomonas aeruginosa* at a concentration of 1200 µg/cup.

The methanolic extract showed zones of inhibition against tested bacterial strains and it showed maximum zones of inhibition (16mm) on *Pseudomonas aeruginosa* at a concentration of 1200 µg/cup.

Heliotropium indicum

All the tested extracts at different concentrations have shown significant antibacterial activity against Gram -ve organisms than Gram +ve organisms along with standard drug.

Hexane extract produced very low zones of inhibition against bacterial strains compared with other extracts and it showed highest zone of inhibition (8mm) against gram +ve bacterial strains at a concentration of 1200 µg/cup.

Ethyl Acetate and Hydro-alcoholic (Ethanol 70% v/v) extracts showed moderate zones of inhibition on tested bacterial strains. Ethyl Acetate extract showed maximum zone of inhibition (14mm) on *Klebsiella pneumoniae* at a concentration of 1200µg/cup and the hydro-alcoholic extract showed maximum zone of inhibition (13mm) on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at a concentration of 1200 µg/cup.

The methanolic extract showed better activity against tested bacterial strains compared to other extracts and showed maximum zones of inhibition (14mm) on *Bacillus megaterium* at a concentration of 1200 µg/cup.

The three plant extracts showed antibacterial activity against selected Gram $_{+Ve}$ and Gram -ve bacteria. Among the extracts methanolic and Hydro-alcoholic extracts showed better antibacterial activity.

A Summary and conclusions drawn are described in Chapter IX. References indicating the sources of information are given at the end of the thesis.

Thus, the results of the present investigation clearly indicated that the selected herbal drugs possess good anti-inflammatory, hepatoprotective and antibacterial activities lead to support the folkloric claims scientifically.